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**Relationship between biological, pathological and toxicological parameters and the cause of death in harbour porpoises (*Phocoena phocoena*) stranded on the coast of Belgium and northern France**

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The Marine Animals Research & Intervention Network (MARIN) investigated the cause of death of marine mammals stranded on the coastline of the southern North Sea (Belgium and northern France) or as bycatch delivered by fishermen. The aim of the study was to compare biological, pathological and toxicological parameters involved in the cause of death (traumatic or infectious) of harbour porpoises (*Phocoena phocoena*) collected between 1990 and 2008. In total, 520 porpoises were necropsied and sampled. Two causes of death appeared to prevail: firstly infectious diseases 191 animals had severe parasitosis and pneumonia, and secondly bycatch in fishing nets: 120 porpoises had died of trauma, mostly due to bycatch in fishing gear. Most strandings and bycatches occurred in winter, and there were more males than females. The majority of

stranded bycaught animals were juveniles. Compared to bycaught porpoises, animals that had died of an infectious process had a thinner blubber layer (emaciation) and the histological investigation showed a marked lymphoid depletion (spleen, thymus and lymph nodes). Nevertheless, many of bycaught porpoises that could serve as a "control" population also presented evidence of emaciation and infectious diseases. This confirms the need to carry out a complete necropsy to evaluate their health status regardless of their cause of death. The sum of PCBs was determined in the blubber of selected individuals with higher concentrations in males than females and also higher levels in adults compared to juveniles. Finally, the animals that had died of infectious disease were more contaminated than bycaught porpoises. Our observations are very similar to results previously reported from neighbouring countries.

Keywords: Harbour porpoises, *Phocoena phocoena*, cause of death, PCBs, health status

#### Introduction:

The multidisciplinary research group MARIN (Marine Animals Research and Intervention Network) deals with marine mammal strandings along the coasts of Belgium and Northern France. The aims of MARIN is to identify the causes of death and monitor the health status of marine mammals. This work includes determining concentrations of chemical contaminants in tissues and finding correlations between lesions and toxicological data and with human activities such as navigation and commercial fishing (Jauniaux et al. 2002). Indeed, the North Sea is heavily polluted with contaminants such as heavy metals and persistent organic pollutants (POP) like polychlorinated biphenyls (PCBs), which accumulate in marine mammals as top predator of the North Sea ecosystem. Harbour porpoises (*Phocoena phocoena*) are the most common cetaceans in the North Sea (Hammond et al. 2002). The most frequent causes of death of North Sea porpoises are accidental capture in fishing nets (by catch) and infectious diseases, mostly bronchopneumonia (Jepson et al. 2000, Siebert et al. 2001, Jauniaux et al. 2002). There is evidence suggesting that PCBs may predispose to infectious diseases as higher concentrations were found in animals that died of infectious disease compared with healthy harbor porpoises that died of acute physical trauma (Jepson et al. 1999, Jepson et al. 2005).

The aim of the study was to compare biological, pathological and toxicological parameters involved in the cause of death (traumatic or infectious) of harbour porpoises collected between 1990 and 2008.

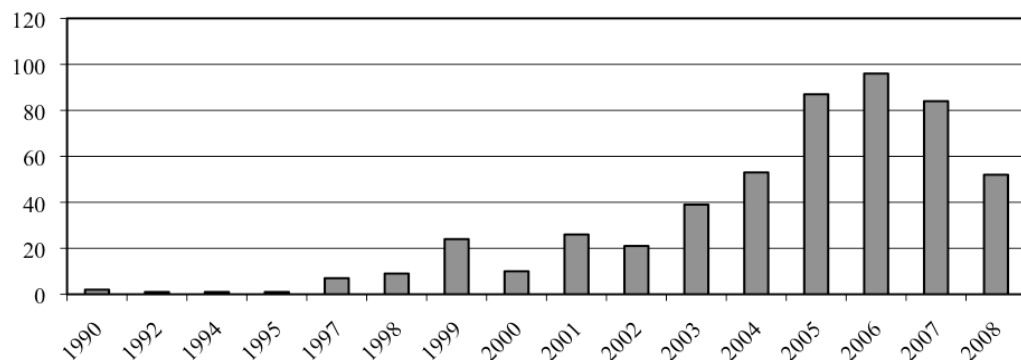
#### Material and methods

Between 1990 and 2008, 520 porpoises stranded on the coastline of the southern North Sea (Belgium and northern France) or were caught incidentally in fishing nets. They were necropsied at the Department of Veterinary Pathology of the University of Liege (Jauniaux & Coignoul 2002). Three age categories based on body length were recognized: neonatal ( $\leq 90$  cm), immature (91-130 cm) and mature ( $>130$  cm). The porpoises were measured, weighed and the blubber thickness was measured at the cranial insertion of the dorsal fin. After external examination, the abdominal and thoracic cavities were opened and the skull was sown longitudinally to expose the brain. Samples were collected for histopathology, microbiology, life history, food study and toxicology. For toxicology, previously published methodology was applied (Weijjs et al. 2009c, b).

#### Result and discussion

The annual distribution of stranding was irregular (Figure 1) with a significant rise since 2000 that reaches a maximum in 2006.

Figure 1: Annual distribution of harbour porpoise stranding



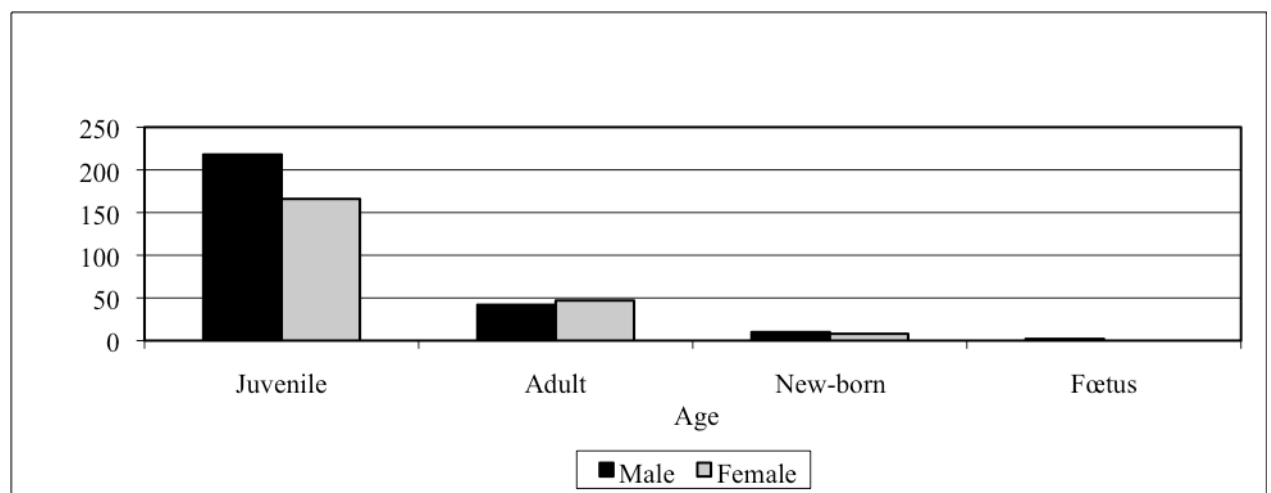
The significant rise of harbour porpoise strandings, also reported for neighboring countries (The Netherlands, United Kingdom) may be linked to the southward shift of the population (Hammond et al. 2002, Hammond 2006). The 2006 peak can be explained by a higher

incidence of capture during that year.

Two causes of death appeared to prevail: firstly infectious diseases – 191 animals had severe parasitosis and pneumonia; and secondly bycatch in fishing nets – 120 had animals died of trauma, mostly due to bycatch in fishing gear. For 209 animals, either the cause of death could not be determined mostly due to the advanced decomposition of carcasses or other causes of death were diagnosed (tumor, starvation,...). Parasitosis was characterized by severe parasite infestation, mostly nematodes, in airways, pulmonary blood vessels and the right ventricle, pulmonary parenchyma and the inner ear. Bronchopneumonia was characterized by large areas of pulmonary consolidation with haemorrhagic or purulent fluid oozing from the parenchyma. The most frequent observations leading to the bycatch diagnosis were the presence of net marks, evidence of amputation, extensive pulmonary congestion and oedema, good nutritional status and absence of evidence of severe disease. However, those observations were not always present simultaneously and frequently parasitosis and mild to severe infectious lesions were also present on bycaught porpoises.

Most strandings occurred in winter with predominance of bycatches in February and March. There were more males (57%) than females (42%) and more juveniles (77%) than adults (22%) (Figure 2).

Figure 2: Age and sex distribution

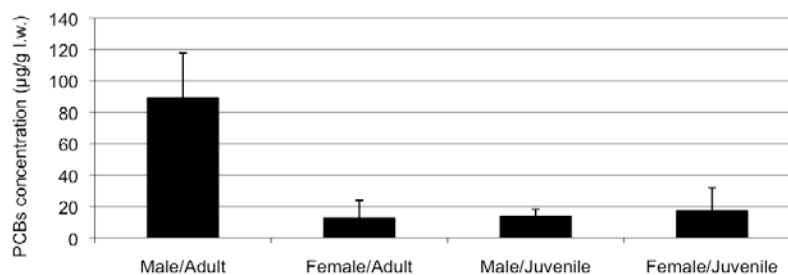


Most of harbour porpoises had an empty stomach, although there was a higher incidence in harbour porpoises dying of an infectious disease. The blubber thickness

was thicker for the bycaught porpoises ( $18 \pm 7$  mm) compared with porpoises dying of an infectious disease ( $12 \pm 15$  mm). A similar relationship between the blubber thickness was reported for porpoises stranded on the coastlines of the United-Kingdom and Germany, porpoises dying of an infectious process were more emaciated (Siebert et al. 2001, Jepson et al. 2005). The majority of stranded bycaught animals were juveniles. For animals that had died of an infectious process, the histological investigations showed a marked lymphoid depletion (spleen, thymus and lymph nodes) compared to bycaught porpoises. The origin of the lymphoid depletion can be linked to the emaciation or associated with the highest concentrations of PCBs found in some individuals (see below).

The sum of PCBs was determined in the blubber of selected individuals ( $n = 18$ ). The concentration (Figure 3) was higher in adult males ( $61.73 \pm 44.09$   $\mu\text{g/g lw}$ ) than in adult females ( $15.24 \pm 12.64$   $\mu\text{g/g lw}$ ) and juveniles of both sexes ( $15.46 \pm 10.37$   $\mu\text{g/g lw}$ ).

Figure 3: Concentrations of sum of PCBs according to age and gender



The highest concentrations of sum PCBs were often reported in males and in adults (Weijs et al. 2009a). The age variation is explained by the process of bioaccumulation, while the gender variation is explained by PCBs transfer between mother and calf during the gestation and the lactation. Finally, the animals that had died of infectious disease ( $49.91 \pm 43.16$   $\mu\text{g/g lw}$ ) were more contaminated than bycaught porpoises. ( $12.35 \pm 4.12$   $\mu\text{g/g lw}$ ). A similar association between concentrations of PCBs and the cause of death was also reported for harbour porpoises stranded on the coastlines of the United-Kingdom and Germany (Jepson et al. 1999, Jepson et al. 2005).

## Conclusions

Infectious process and the capture in fishing nets are the two most frequent causes of death representing respectively 61% and 39% when the cause of death is determined.

The most frequent observations reported on porpoises dying of infectious process were severe parasitosis, acute pneumonia, emaciation and lymphoid depletion. Often, these animals also had an empty stomach and the highest concentrations of PCBs. Nevertheless, even if the relationship between those observations is apparent and has been reported as well for porpoises stranded in other parts of the North Sea, the mechanism? of such association is not entirely understood. High concentrations of PCBs can predispose to immunodepletion, thereby increasing the risk of infectious disease. On the other hand, emaciation can play a similar effect. .

Finally, bycaught porpoises being a random sample of the population they can serve as a "control". These animals also presented evidence of emaciation and infectious diseases, thus confirming the need to carry out a complete necropsy to evaluate their health status.

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